

Case Report

Surgical wound infection by mannitol-nonfermenting *Staphylococcus aureus* after lumbar microdiscectomy

Vincenzo Savini¹, Raffaele Nigro², Roberta Marrollo¹, Ennio Polilli¹, Irma Campitelli¹, Roberto Buonaguidi², Paolo Fazii¹, Edoardo Carretto³

¹Clinical Microbiology and Virology, Spirito Santo Hospital, Pescara (PE), Italy; ²Neurosurgery, Spirito Santo Hospital, Pescara (PE), Italy; ³Clinical Microbiology Laboratory, IRCCS Arcispedale Santa Maria Nuova, Reggio Emilia (RE), Italy

Received February 20, 2014; Accepted March 7, 2014; Epub April 15, 2014; Published May 1, 2014

Abstract: Purulent infection of a surgical wound developed after discectomy, and a mannitol-nonfermenting *Staphylococcus aureus* isolate was cultivated as the etiologic agent. Nonfermenting *S. aureus* strains are exceedingly rare and may be erroneously mistaken and dismissed as contaminants. This report then emphasizes that pure and massive cultures must be carefully evaluated, even if preliminary examination does not suggest a pathogenic organism. Also, although mannitol-negative, the studied strain was correctly detected as *S. aureus* by both the-FISH test (AdvanDx, USA) and the Liofilchem 'Chromatic Staph aureus', highlighting that additional diagnostic methods may support recognition of uncommon, nonfermenting *S. aureus* strains in the daily practice.

Keywords: Mannitol, *Staphylococcus aureus*, surgical wound

Case report

A 43-year-old man with a three-year history of back pain radiated to the left side of the left leg presented to the neurosurgery Department of the Pescara Civic Hospital, Italy. His remote pathological anamnesis was unremarkable, while magnetic resonance (MR) imaging showed a left posterolateral disc herniation L4-L5. Conservative therapy, including limited activity and analgesics, was unsuccessful in relieving symptoms; the patient then underwent microdiscectomy and was discharged two days after surgery. Two weeks later he presented for suture removal and, in this occasion, the wound appeared markedly flushed and swollen, with two purulent discharges from the lesion upper and middle third (**Figure 1**). The patient also referred fever (up to 39°C) and was therefore newly admitted to hospital. Suture was removed and the wound exudate collected for culture. Empirical treatment with topical rifampin and iodoformic gauzes (once a day) was started, concomitantly, pending microbiology results.

After 24 h incubation, a mannitol-nonfermenting, catalase- and Gram-positive coccoid iso-

late grew on mannitol salt and trypticase soy agar (containing 5% sheep blood) (media by Liofilchem®, Italy) (**Figure 2**), while no other organisms were observed either aerobically or under anaerobic conditions. Although the isolate (which still did not ferment mannitol after 48 h incubation) could be initially labeled as a coagulase-negative *Staphylococcus* (CoNS), it grew as pink-mauve colonies (**Figure 2**) on the Chromatic Staph aureus (Liofilchem®), a chromogenic medium designed to presumptively detect *S. aureus* from biological samples. Accordingly, the isolate was found to coagulate rabbit plasma, it was identified as *S. aureus* by the Vitek2 GP card (bioMérieux, France) and, as a confirmation, *spa* gene-positivity was detected through the GeneXpert technology (Cepheid, US). Again, the *Staphylococcus* Quick-FISH test (AdvanDx, USA), performed on the isolate, gave a brilliant green fluorescence (that is specific to *S. aureus*), and a home-made PCR based on the analysis of the polymorphism of the *nuc* gene [1] definitively confirmed identification. The strain was stored into the internal laboratory collection under the accession number Sa90. To investigate whether the patient was an Sa90 carrier, furthermore, nasal swabs were

Purulent infection of a surgical wound

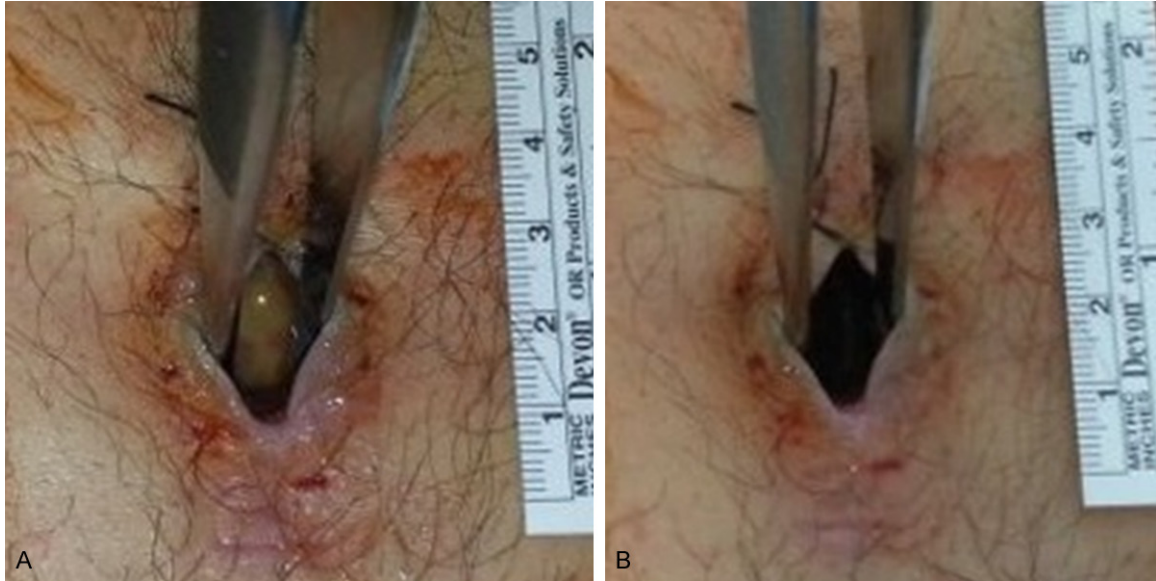


Figure 1. A: The inflamed surgical wound described in the text (purulent material is visible inside the lesion); B: The sterilized wound under antibiotics.

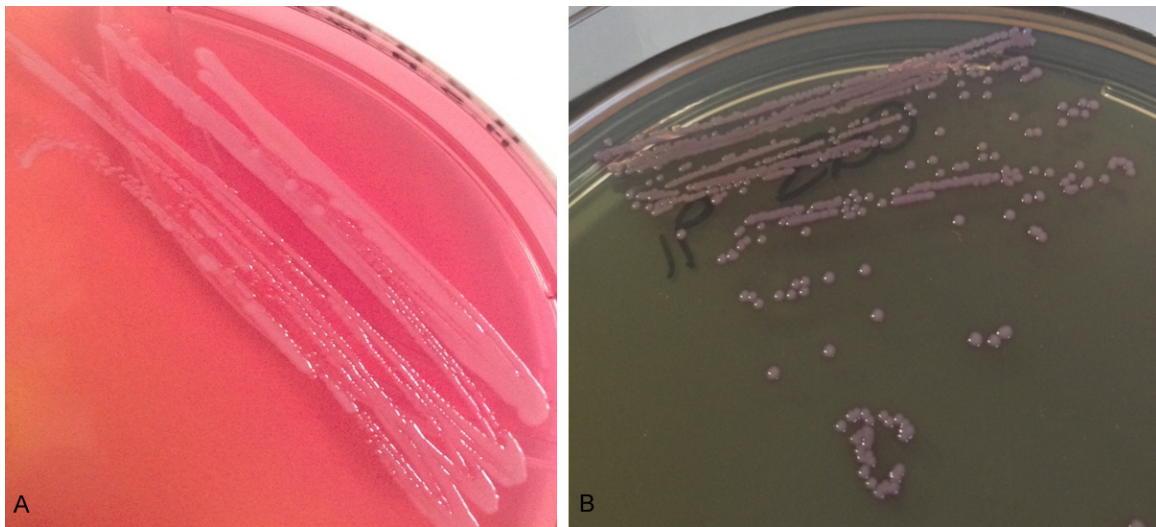


Figure 2. A: Mannitol-negative Sa90 colonies after 48 h incubation on mannitol salt agar (Liofilchem®, Italy); B: Pink-mauve-pigmented Sa90 colonies on the Chromatic Staph aureus (Liofilchem®, Italy).

performed and, interestingly, revealed colonization by the same strain (the nasal isolate was named Sa91); in fact, the two organisms showed a unique molecular fingerprint by using both the semiautomated repetitive element palindromic PCR (rep-PCR) (DiversiLab, bio-Mérieux) and the random amplified polymorphic DNA (RAPD) technique [2]. Additionally, both were found not to harbor the *mecA* gene (with the GeneXpert), and to exert *in vitro* susceptibility to macrolides, clindamycin, gentami-

cin, levofloxacin, rifampin and cotrimoxazole (antibiotic susceptibility testing performed by Liofilchem® MIC test strips). The patient was discharged from hospital under oral rifampin plus cotrimoxazole (600 mg/die and 800/160 mg twice a day, respectively), with an indication to return for wound evaluation and medication on alternate days. At clinical examination, infection signs disappeared within a 7-day course of antibiotics, while subsequent cultures performed after treatment were found to be nega-

Purulent infection of a surgical wound

tive; healing by second intention was finally achieved in a month.

Mannitol salt agar (MSA) was designed in 1945 to obtain presumptive recognition of pathogenic staphylococci from clinical samples, and its use relies on the ability of *S. aureus* to ferment mannitol, thus growing as yellow-pigmented colonies [3].

Mannitol nonfermenting *S. aureus* strains are presumed to be rare, accounting for 2.2% of all isolates [3]; nevertheless, these phenotypes may go underrecognized, as they mimic CoNS, leading to underestimation [3, 4]. Mechanism for non-fermentation is unclear, though it is reported that genetic mutations may make certain strains lack the ability to ferment; it is however unexplained why some mannitol-nonfermenting isolates (on MSA) may produce acid (from mannitol) on the API STAPH system (bio-Mérieux). Additionally, a catalase-negative isolate has been described in the literature to produce acid aerobically, while it did not anaerobically [3]. Mannitol-negative isolates have been identified as the agents of food poisoning, wound infections, and bacteremias [3, 4], then we may add to knowledge of these uncommon variants' pathogenicity and want to emphasize that daily diagnostics cannot be too heavily based on biochemical markers only [4]. Conversely, a massive growth as a pure culture must be carefully considered, case-by-case, even if preliminary morphology examination seems not to suggest a potentially pathogenic organism.

Again, this communication further highlights that nasal *S. aureus*-carriers are at an increased risk for postoperative infections and decolonization at hospital admission might therefore reduce the incidence of health care-associated diseases [5, 6]. Finally, the abovementioned Liofilchem® chromogenic medium suggested us a correct, preliminary identification, then its performance might be further investigated with a wider number of clinical isolates, as it may be a promising tool to easily detect mannitol-negative strains.

Disclosure of conflict of interest

None to declare.

Address correspondence to: Vincenzo Savini, Clinical Microbiology and Virology, *Spirito Santo* Hospital, via Fonte Romana 8, CAP 65124, Pescara (PE), Italy. Tel: 0039 340-7379737; E-mail: vincenzo_savini@libero.it

References

- [1] Sasaki T, Tsubakishita S, Tanaka Y, Sakusabe A, Ohtsuka M, Hirotsuki S, Kawakami T, Fukata T, Hiramatsu K. Multiplex-PCR method for species identification of coagulase-positive staphylococci. *J Clin Microbiol* 2010; 48: 765-769.
- [2] Damiani G, Telecco S, Comincini S, Sironi M, Carretto E, Marone P. Comparison of an improved RAPD fingerprinting with different typing methods for discriminating clinical isolates of *Staphylococcus* spp. *Eur J Epidemiol* 1996; 12: 163-169.
- [3] Shittu A, Lin J, Morrison D. Molecular identification and characterization of mannitol-negative methicillin-resistant *Staphylococcus aureus*. *Diagn Microbiol Infect Dis* 2007; 57: 93-95.
- [4] Haga S, Hishinuma I, Itoh I, Kumagai N, Eguchi H, Tanaka M. Food poisoning caused by mannitol nonfermenting *Staphylococcus aureus*: case report. *Jpn J Infect Dis* 2007; 60: 145-146.
- [5] Weinstein HJ. The relation between the nasal-staphylococcal-carrier state and the incidence of postoperative complications. *N Engl J Med* 1959; 260: 1303-1308.
- [6] Bode LG, Kluytmans JA, Wertheim HF, Bogaers D, Vandenbroucke-Grauls CM, Roosendaal R, Troelstra A, Box AT, Voss A, van der Tweel I, van Belkum A, Verbrugh HA, Vos MC. Preventing surgical-site infections in nasal carriers of *Staphylococcus aureus*. *N Engl J Med* 2010; 362: 9-17.